Journal of Medicine, Physiology and Biophysics
ISSN 2422-8427 An International Peer-reviewed Journal
Vol.34, 2017
www.iiste.org

Prevalence and Multi Drug Resistance Patterns of Nasal Carriage Staphylococcus aureus in Dairy Workers in and around Asella Town, Arsi Zone, South Eastern Ethiopia

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Abstract
A cross-sectional study was carried out from November 2012 - May 2013 to estimate the prevalence and multi drug resistance patterns of nasal carriage *Staphylococcus aureus* and its putative risk factors among dairy farm workers in and around Asella town, Arsi Zone, South Eastern Ethiopia. A total of 96 nasal swab samples were collected from volunteer dairy farm workers using convenient sampling method. The collected samples were subjected to bacteriological examinations, using different techniques according to standard procedures, for isolation and identification of *Staphylococcus aureus*. *Staphylococcus aureus* isolates were identified from 39.58% (38/96) of nasal swab samples. Multivariate logistic regression analysis of the effect of different risk factors on the prevalence of nasal carriage *Staphylococcus aureus* revealed that; male individuals (OR = 5.773, 95%CI: 1.727, 19.296) and poor milking hygiene scores (OR= 4.805, 95%CI: 1.651, 13.985) were more likely to be colonized with nasal carriage *S. aureus* than their counter parts. The isolates of *Staphylococcus aureus* were tested for antimicrobial susceptibility test by disc diffusion method. Susceptibility to methicillin was phenotypically determined based on sensitivity of isolates to cefoxitin and oxacillin. The highest rate of susceptibility was to vancomycin (97.4%) followed by gentamycin (96.2%), chloramphenicol (85.3%), clindamycin (82.6%), erythromycin (71.5%) and streptomycin (69.6%). Whereas, the highest rate of resistance among the isolates was against penicillin G (83.7%) followed by ampicillin (71.7%), cefoxitin (60.8%), oxacillin (56.1%), tetracycline (54.9%), amoxicillin-clavulinic acid (42.4%) and trimethoprim-sulfamethoxazole (39.5%). Of the total nasal carriage *Staphylococcus aureus* isolates, 60.8% were MRSA and 55.3% were MDRSA. The presence of multidrug resistant isolates in nasal swabs among dairy farm workers demonstrates the importance of the choice and appropriate use of antimicrobial agents. Regular antimicrobial sensitivity testing and recognizing the appropriate pattern of antibacterial resistance could pave the way for optimized antibiotic prescription in order to prevent resistance to newly developed antibiotics.
Keywords: *Assella,* dairy workers, multidrug resistance, nasal carriage *Staphylococcus aureus*, prevalence.

INTRODUCTION

Staphylococcus aureus is recognized worldwide as a leading pathogen causing many serious diseases in dairy and healthcare surroundings. Approximately 20–30% of human population carries *S. aureus*, in their anterior nares (Graham et al., 2006). Nasal carriage of Staphylococcus aureus plays a key role in the development of S aureus infections. The reservoir for *S aureus* skin infection is the anterior nares. Nasal carriage is an epidemiologic biomarker of *S. aureus* exposure associated with increased risk of infection (Kluytmans et al., 1997; Wertheim et al., 2005; Safdar and Bradley, 2008), and is used widely in research to assess human exposure to livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) (Smith and Pearson, 2011).

Both healthy carriers and infected individuals can transmit *S. aureus* directly or indirectly to others. In bovines, *S. aureus* mainly causes mastitis with subsequent contamination of milk and dairy products (Oliver et al., 2005). Studies have predicted human-to-bovine transmission by recovering *S. aureus* clones from cattle that are closely related to those obtained from humans (Roberson et al., 1994). Moreover, livestock can also act as a reservoir for the emergence of new human bacterial clones with potential for pandemic spread, highlighting the potential role of surveillance and biosecurity measures in the agricultural setting for preventing the emergence of new human pathogens (Spoor et al., 2013).

Among livestock farmers, occupational contact with cattle is a risk factor for nasal colonization with MRSA ST398 (Adesiyun et al., 1998; Moodley et al., 2008; van Cleef et al., 2011; Antoci et al., 2013; Lim et al., 2013; Alba et al., 2015). Intensity of animal contact has been identified as a risk factor for nasal carriage of LA-MRSA among workers (Vandendriessche et al., 2013; Graveland et al., 2011) as has MRSA contamination in the occupational environment (Dorado-Garcia et al., 2013).

Both human and non-human antimicrobial usage may result in increased occurrence of bacterial resistance (Anderson *et al*., 2003). Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water

and food (Normanno et al., 2007). The problem of antibiotic resistant *S. aureus* is extremely challenging (D'Agata, 2002). *S. aureus* has become resistant to many commonly used antibiotics. Multidrug Resistant *S. aureus* (MDRSA) isolates have been emerged in various parts of the world. Resistance to β-lactams and other antibiotic groups is associated with longer hospitalization and more cost of treatment (Kim et al., 2001). In 1991, the prevalence of penicillin resistant *S. aureus* was 91% while methicillin resistant *S. aureus* (MRSA) was 29% (Moreira and Daum, 1995). *S. aureus* species are becoming increasingly resistant to methicillin and multiple other drugs. In 1990s, nearly all studies reflected the MRSA species (Struelens, 1994). The prevalence of MRSA progressively increased thereafter; however, great geographic variations exist. Furthermore, the emergence of community acquired methicillin-resistant *S. aureus* (MRSA) has become an important challenge for the treatment of staphylococcal infections due to its high virulence and emerging antibiotic resistance of this kind of *S. aureus* (Kaplan et al., 2005).

Staphylococcus aureus isolates of human and animals have been extensively analyzed in developed countries but there is only sparse information on the nasal carriage *Staphylococcus aureus* isolates of human from Africa (Schaumburg et al., 2011). In particular, little is known about *Staphylococcus aureus* infection and asymptomatic carriers in human and animal in Ethiopia except pronounced reports on the effect of *Staphylococcus aureus* infection as number one cause of bovine mastitis which resulted in great economic losses (Mekonen et al., 2005).

Despite rapid improvement in antimicrobial therapy, there are still great difficulties in the treatment of staphylococcal infections. Multidrug Resistant *S. aureus* (MDRSA) isolates have been emerged in various parts of the world (Normanno et al., 2007). To elucidate mechanisms underlying the alarming global trends in antimicrobial resistance, careful characterization of antimicrobial resistance patterns among bacteria from human, particularly nasal carriage *S. aureus* is paramount as it is a substantial source of human infections. Determination of the prevalence of nasal carriage *Staphylococcus aureus* among dairy workers and recognizing the appropriate pattern of antibacterial resistance could pave the way for optimized antibiotic prescription in order to prevent resistance to newly developed antibiotics.

In Ethiopia, there are a limited number of publications on the epidemiological aspects of infections in both animals and humans; only a few reports have been published on nasal carriage *S. aureus* and its multidrug resistance profile from individuals who have close contact with animals (Mekuria et al., 2013). Due to the noticeable increase in antimicrobial resistance, determination of antibiotic susceptibility profile is judicious for decolonization and treatment of *S. aureus* infections. To our knowledge, no prior studies have evaluated *S. aureus* nasal carriage and its multi drug resistance patterns among dairy workers in Arsi zone in general and in and around Asella town in particular. Therefore, this study was aimed to determine the prevalence of nasal carriage *S. aureus* from dairy farm workers who had close contact with animals and the resistance rates of *S. aureus* isolates against various antimicrobials commonly used in Ethiopia.

MATERIALS AND METHODS

Study design and description of the study area

A cross sectional study was conducted from October 2012 to May 2013 on volunteer dairy workers in and around Asella town of Arsi zone, Oromia Regional State, South Eastern Ethiopia. Asella town is located at a distance of 175km south east of Addis Ababa at 7°57'N and 39°7'E with an altitude of 502-4130 meters above sea level and annual rainfall of the area ranges from 200-400 mm with mean annual temperature of 22.5°C. It is one of the highly populated areas in Ethiopia with estimated human population of 2,521,349. Agricultural production system in the vicinity of Asella town is mixed crop and livestock farming. Dairy farming using improved breeds is a common practice in the study area.

Study participants

An informed consent was obtained from the dairy farm workers for participation in the study following explanation about aims of the investigation. An interview was performed with the workers and a questionnaire was filled by interviewer. The questionnaires included the associated risk factors for nasal colonization with MDRSA, such as age, gender, duration of animal contact/working hours per week in dairy farms**,** farm hygiene practices associated with milking and educational level.

Nasal sample collection

Nasal swabs were collected from 96 volunteer dairy farm workers who were in close contact with the cattle in and around Asella town. Great care was taken to avoid contamination of micro flora indigenous to the skin and mucous membranes, growth of which may lead to inappropriate diagnosis and therapy. All workers included in the study were healthy and did not have any medical complication at the time of sampling. Nasal specimens were collected from workers using sterile, plastic capped cotton-tipped swab stick, transport tube with Stuart medium. The swab was circled through both nostrils consecutively. The swabs were stored in Stuart transport medium and were immediately transported to the laboratory in a cool box on ice.

Isolation and identification of *Staphylococcus aureus*

Nasal swabs were inoculated into tryptone soya broth (TSB, Oxoid Ltd., Basingstoke, Hampshire, UK), supplemented with 7 % (w/v) sodium chloride, and incubated aerobically at 35°C for 24 hours. After overnight incubation at 37°C, 100 µl of the culture broth were transferred into a selective Mannitol-Salt-Agar (Oxoid, UK). Then, 100 µL of the broth was inoculated on to blood agar (Oxoid Ltd., Basingstoke, Hampshire, UK) containing 7% sheep blood and the plates were incubated aerobically at 35°C and examined after 24hours of incubation for growth (Lee et al., 2004). The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology, colony morphology, pigmentation and hemolytic pattern on blood agar and other environment from which the bacterium were isolated. After the incubation, each different colony was examined macroscopically (colony morphology, haemolysis, pigment production) and microscopically (Gram staining). Identification of growing colonies was achieved using standard conventional methods. For this purpose, indole, oxidase, catalase, slide and tube coagulase with rabbit plasma, Voges-Prouskauer, anaerobic fermentations of glucose, lactose, sucrose, maltose and mannitol were used to identify *S. aureus* (Holt et al., 1994; Quinn et al., 2004).

Antimicrobial susceptibility testing

The *Staphylococcus aureus* isolates were tested for anti-microbial susceptibility by disc diffusion method (Quinn et al*.,* 2004; CLSI, 2011). Antimicrobials of veterinary and human health relevance were considered. Antimicrobial agents from different antibiotic classes were used. The following antibiotics (Oxoid, Hampshire, England) were used for testing: ampicillin (10µg), vancomycin (30µg), gentamycin (10µg), erythromycin (15µg), clindamycin (10µg), tetracycline (30µg), oxacillin (1ug), amoxacillin (25µg), chloramphenicol (30µg), trimethoprim-sulfamethoxazole (25µg), cefoxitin (30 µg), and penicillin G (10µg). In brief, the isolates were inoculated in tryptone soya broth (TSB) and incubated at 37°C for 24hrs. The turbidity of the suspension was adjusted to obtain turbidity visually comparable with that of 0.5 McFarland standards. Muller-Hinton Agar (MHA) plate was prepared and a sterile cotton swab was dipped into the suspension and swabbed on the surfaces of Muller-Hinton Agar plate. Then, the antibiotic discs were placed on the agar plate using sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates were read 24hrs after incubation at 37^{O}C under aerobic condition. The isolates were classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards (CLSI, 2011) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacturer's instructions by measuring the zone of inhibition around the antibiotic disc. Intermediate results were considered resistant (Huber *et al*., 2011). Multiple drug resistant *Staphylococcus aureus* (MDRSA) was defined as those resistant to at least three different antibiotics (Magiorakos et al., 2012).

Quality control

Confidence in the reliability of test results is increased by following adequate quality assurance procedures, and the routine use of control 3503 strains, *S. aureus* ATCC25923 as a positive control and *Escherichia coli* ATCC-25922 as a negative control (for culture on MSA) were taken as an important part of quality control for culture and antimicrobial susceptibility test. Thus, quality control microorganisms yielded values within the established ranges, indicating that the test was performed in a satisfactory manner.

Detection of MRSA

Cefoxitin is a potent inducer of the mecA regulatory system. It is being recommended for detection of methicillin resistance in *S. aureus* (MRSA) when using disk diffusion testing. Results of cefoxitin disc diffusion test is in concordance with the PCR for mecA gene, and thus the cefoxitin disk diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA (Anand et al.2009; Broekema et al., 2009; Fernandes et al., 2005).

Statistical Analysis

Data were analyzed by STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). Descriptive results were defined using frequencies and percentages. Prevalence was calculated as a percentage value. The association between the independent factors and response variable was evaluated using the Chi-square test ($\chi 2$). Multivariate logistic regression analyses were used to analyze the effects of different potential risk factors on the prevalence of *Staphylococcus aureus* nasal colonization/infection. Odds ratio (OR) was utilized to measure the degree of association between potential risk factors with prevalence of *Staphylococcus aureus* nasal colonization. The 95% confidence interval and a p-values < 0.05 were considered statistically significant.

RESULTS

Out of the total nasal specimens of individuals working in dairy farms tested for the presence of *S. aureus* nasal carriage, 38/96 (39.58%) were tested positive for *S. aureus* as depicted inTable 1.

Table 1. Prevalence of *Staphylococcus aureus* nasal carriage isolated from dairy farm individuals

Type of bacteria isolated	Total samples examined	Number negative (%)	Number positive (%)
Staphylococcus aureus	96	58 (60.42)	38 (39.58)

A significant association ($p < 0.05$) was found between the age groups and the isolation rate of nasal carriage *S. aureus* isolates with the higher prevalence rates encountered in the age group ≥ 20 (47.69%, 31/65) . Nasal *S. aureus* carriage prevalence was significantly higher among men in comparison with women (55.93% vs. 13.51%, $p < 0.001$). Duration of animal contact was significantly associated ($p < 0.05$) with the prevalence of *S. aureus* nasal carriage in dairy workers. Milking hygiene scores significantly varied with the percentage of positive dairy workers on the farms ($p<0.01$). Moreover, prevalence of *S. aureus* isolates was statistically significant ($p < 0.05$) with level of education of dairy workers as illustrated in Table 2.

Table 2. Association of study participants and farms characteristics with nasal carriage *S. aureus* prevalence.

Factor	Category	No. examined	No. positive	Prevalence (%)	$\chi 2$ (P value)
Age (in years)	< 20	31	7	22.58	
	≥ 20	65	31	47.69	5.535 (0.019)
Gender	Male	59	33	55.93	17.109 (0.000)
	Female	37	5	13.51	
Working hours per week	< 25 hours	26	6	23.08	
	≥ 25 hours	70	32	45.71	4.062 (0.044)
Milking hygiene scores	Poor	41	24	58.54	10.750 (0.001)
	Good	55	14	25.45	
Educational level	Illiterate	38	21	55.26	6.466 (0.011)
	Literate	58	17	29.31	

Logistic regression analysis of the effect of putative risk factors on the prevalence of *S. aureus* nasal colonization is depicted in Table 3. Accordingly, gender and milking hygiene scores significantly enhance the risk for nasal carriage *S. aureus* colonization. Hence, male gender (OR= 5.773, 95%CI: 1.727, 19.296) and poor milking hygiene scores (OR= 4.805, 95%CI: 1.651, 13.985) were more likely to be colonized with nasal carriage *S. aureus* than their counter parts.

Table 3: Multiple logistic regression analysis to predict the putative risk factors associated with nasal carriage *S.aureus* isolates.

		S. aureus test result	Odds ratio		
Factor	Category	Prevalence (%)	COR (95%CI)	AOR (95% CI)	P value
Age	< 20 years	22.58	1	1	
	≥ 20 years	47.69	3.126 (1.182, 8.267)	3.315 (0.984, 11.175)	0.053
Gender	Female	13.51	1	1	
	Male	55.93	8.123 (2.776, 23.766)	5.773 (1.727, 19.296)	0.004
Working hours/week	< 25 hours	23.08	1	1	
	≥ 25 hours	45.71	2.807 (1.006, 7.834)	3.045 (0.882, 10.509)	0.078
Milking hygiene scores	Good	25.45	1	1	
	Poor	58.54	4.134 (1.735, 9.853)	4.805 (1.651, 13.985)	0.004
Educational level	Illiterate	55.26	1	1	
	Literate	29.31	2.979 (1.269, 6.995)	1.626 (0.572, 4.624)	0.362

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

All the isolates of nasal carriage *Staphylococcus aureus* were tested for susceptibility to panels of 13 antimicrobial agents using disc diffusion assay as illustrated in Table 4. Of the entire antibiotics used in this study, the highest rate of susceptibility was to vancomycin (97.4%) followed by gentamycin (96.2%), chloramphenicol (85.3%), clindamycin (82.6%), erythromycin (71.5%) and streptomycin (69.6%). Whereas, the highest rate of resistance among the isolates was against penicillin G (83.7%) followed by ampicillin (71.7%), cefoxitin (60.8%), oxacillin (56.1%), tetracycline (54.9%), amoxicillin-clavulinic acid (42.4%) and trimethoprim-sulfamethoxazole (39.5%).

Table 4. Antimicrobialresistance profiles of *S. aureus* isolated from mastitic milk (N = 112).

Antibiotics tested	Susceptible (%)	Intermediate (%)	Resistance (%)
Ampicillin	24.8	3.5	71.7
Vancomycin	97.4	-	2.6
Gentamycin	96.2	-	3.8
Erythromycin	71.5	5.4	23.1
Clindamycin	82.6	-	17.4
Tetracycline	40.5	4.6	54.9
Oxacillin	43.9	-	56.1
Amoxicillin-clavulinic acid	55.3	2.3	42.4
Chloramphenicol	85.3	1.8	12.9
Streptomycin	69.6	-	30.4
Trimethoprim-sulfamethoxazole	54.2	6.3	39.5
Cefoxitin	39.2	-	60.8
Penicillin G	8.7	7.6	83.7

Susceptibility to methicillin was phenotypically determined based on sensitivity of isolates to cefoxitin and oxacillin. Cefoxitin can detect some isolates not recognized by oxacillin and testing isolates with both drugs has been recommended (CLSI, 2008). Significant proportion of *Staphylococcus aureus* nasal carriage isolates were resistant to cefoxitin (60.8%), implying they were methicillin resistant *Staphylococcus aureus* (MRSA). CLSI recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine resistance against methicillin for *S. aureus* (CLSI, 2008). In the present study, all the isolates of *Staphylococcus aureus* resistant to oxacillin were also resistant to cefoxitin disc.

Multi-drug resistant *Staphylococcus aureus* (MDRSA) in this study was taken as resistance to three or more of the 13 antimicrobial drugs tested. Accordingly, the rate of MDRSA isolates that were resistant to at least three different antimicrobials in the present study was 55.3% (Table 5).

Table 5. Percentage of resistant *S. aureus* isolates to antimicrobials from dairy farm workers (n = 38).

Number of antimicrobial discs	Number of resistant isolates	Percentage
One	9	23.6
Two	8	21.1
MDRSA	21	55.3

MDRSA: Multi-drug resistant *Staphylococcus aureus*

DISCUSSION

Ninety six dairy workers were enrolled in this study. The prevalence of nasal colonization with *S. aureus* was 39.58%. This is comparable with the previous findings of Ghazvini and Hekmat (2007) and Ghasemian et al. (2010) who reported a rate of 36.9% and 40.5%, respectively. However, the present finding is relatively lower than the report of Piraino et al (1993), Tigist et al. (2012) and Sarkar et al. (2014) who disclosed prevalence rate of 50%, 57.8% and 70%, respectively. On the other hand, the result of the present study is higher than the prevalence rate of 13.2% and 26.3% reported by Mekuria et al. (2013) and Erami et al. (2014), respectively. The apparent geographical variation in the prevalence rates of *S. aureus* nasal colonization might reflect differences in socioeconomic status, hygiene practices prevailing in different parts of the country, age, gender and types of test used, that is, sensitivities and specificities of the diagnostic methods used among researchers might also influence the outcome. The relatively higher proportion of these bacteria in dairy workers' nasal swabs might be related to several factors such as poor hygiene practices, duration of animal contact and other individual factors.

The present study revealed that higher prevalence rates recorded in aged individuals and male gender than young and female individuals and significantly associated ($P < 0.05$) with *S. aureus* nasal colonization. Multiple logistic regression analysis revealed that male individuals (OR= 5.773, 95%CI: 1.727, 19.296) had higher odds for acquiring nasal carriage *S. aureus* than female. The current result is in agreement with the work of Saxena et al. (2004) who found a significant correlation between age and nasal carrier state.

Moreover, it was investigated that duration of animal contact was significantly associated with nasal colonization of *S. aureus*. Our study concords with previous report of Armand-Lefevre et al. (2005) who revealed that people working with livestock are at a potential risk of becoming MRSA carriers and hence are at an increased risk of infections caused by MRSA. The presence of MRSA in bovine milk and dairy environments poses potential risk to farm workers, veterinarians and farm animals that are exposed to contaminated cattle (Hanselman *et al*., 2006; Gunaydin *et al*., 2011). The transmission of milk-associated *S. aureus* strains between cows and humans was suggested by Lee (*2003*), whose study showed MRSA in milk samples with comparable antibiotypes as those in humans.

It was observed that higher proportion of nasal carrier *S. aureus* from dairy workers significantly related to poor milking hygiene practices. Workers from poorly managed farm showed higher prevalence than good

managed farm individuals. Multiple logistic regression analysis revealed poor milking hygiene scores (OR= 4.805, 95%CI: 1.651, 13.985) were more likely to be colonized with nasal carriage *S. aureus* than their counter parts. This is in agreement Rowe (1999) who reported that *Staphylococcus aureus* is a contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures. Based on observations made during the collection of samples, improper hygiene and poor farm management practices contributed to the high prevalence of *Staphylococcus aureus* in the nasal swabs of dairy workers. In this study area milk was obtained from animals by washing their hands and/or the utensils and containers used. In certain cases, untreated groundwater was used to wash the containers that were used for milking. This may have contributed to the high level of *S. aureus* isolated. Improving the hygienic conditions of the milking environment and/or utensils may reduce the prevalence of *S. aureus* in milk and prevent its transmission to humans.

The highest level of antimicrobial susceptibility shown by *S. aureus* isolates in this study was observed against vancomycin (97.4%) followed by gentamycin (96.2%), chloramphenicol (85.3%), clindamycin (82.6%), erythromycin (71.5%) and streptomycin (69.6%). This is in accordance with the findings of Tariku et al. (2011) and Nwankwo and Nasiru (2011). The reason why these antimicrobials were less resistant might be that they are not frequently used in the study area in veterinary services, and perhaps in human medicine. Similar suggestion was given by Jaims et al. (2002) that the development of antimicrobial resistance is nearly always as a result of repeated therapeutic and/or indiscriminate use of them.

Moreover, the highest rate of resistance among the isolates was against penicillin G (91.3%) followed by ampicillin (79.7%), cefoxitin (60.8%), oxacillin (56.1%), amoxicillin-clavulinic acid (55.3%), tetracycline (54.9%) and trimethoprim-sulfamethoxazole (50.2%). *Staphylococcus aureus* are frequently resistant to other antibiotic agents in clinical use, including β-lactams, fluoroquinolones, aminoglycosides, rifampin, and mupirocin (Carbon, 2000). The resistance of *S. aureus* to penicillin and cefoxitin may be attributed to the production of beta lactamase enzyme that inactivates penicillin and closely related antibiotics. Resistance to Penicillin G is used as a marker to assess the susceptibility of *S. aureus* isolates against other beta-lactam antibiotics (Waage *et al*., 2002; Pace and Yang, 2006).

With a particular emphasis to tetracycline, the present observation agrees with preliminary finding conducted by Bayhun (2008). This is due to the fact that tetracycline is the most commonly used antimicrobial in the treatment of infections in the livestock sector in Ethiopia. Moreover, tetracycline is widely used as growth factors in veterinary medicine for livestock rearing as well in the treatment of bacterial infection occurring in human medicine (Ardic et al., 2005). Tetracycline is intensively used in animal agriculture and resistance to it is commonly identified in livestock-associated *S. aureus* isolates (Roberts, 2002). Our findings reinforce previous work that identifies tetracycline resistance as a marker of livestock-association in *S. aureus*.

It was observed that large percentages of cefoxitin (60.8%) resistant *S. aureus* were isolated from the study area. Disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA by many reference groups including CLSI (Skov et al., 2003). Therefore, one can easily conclude that these are Methicillin resistant *S. aureus* (MRSA). CLSI recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine resistance against methicillin for *S. aureus* (CLSI, 2008). Similarly, various researchers reported that cefoxitin were more sensitive for the detection of mecA-mediated resistance than oxacillin results (Broekema et al., 2009; Tiwari et al., 2009). Broekema et al. (2009) reported the sensitivity of 97.3 % and specificity of 100 % for the cefoxitin disc. In another study, the sensitivity and specificity rates were found to be 77.3 % and 84.6 % for oxacillin and 98.5 % and 100 % for cefoxitin in MRSA strains (Tiwari et al., 2009). Furthermore, another studies reported that out of the 50 isolates, 28 were found to be methicillin resistant by oxacillin disc diffusion test, 30 were resistant by oxacillin screen agar method, and 32 were resistant with cefoxitin disc diffusion. For these 32 isolates were mecA genes positive (Anand et al.2009; Broekema et al., 2009; Fernandes et al., 2005). According to these findings, we considered that the cefoxitin disc may be used in the routine diagnosis of MRSA strains.

S. aureus strains have developed multidrug resistance worldwide with broad diversity in prevalence rate in different regions (Normanno et al., 2007). In the present observation, 55.3% of *S. aureus* isolates showed multidrug resistance primarily to penicillin G, ampicillin, cefoxitin, oxacillin, amoxicillin-clavulinic acid, tetracycline and trimethoprim-sulfamethoxazole. The present finding is relatively higher than that of Mohamed et al. (2011) and Mekuria et al. (2013) who reported 45% and 45.1% of MDRSA from Saudi Arabia and Addis Ababa dairy farm workers, respectively. However, the current result is lower than the previous work of Barena and Fetene (2003) and Chao et al. (2007) who reported MDRSA at the rate of 80% and 79% among the isolates, respectively. The reasons of this result may be attributed to the production of beta lactamase enzyme; intensive, uncontrolled and prolonged use of and accessibility to these antibiotics without a medical prescription, high use of antibiotics in animal production and multi-resistant strains of microbes in animal farming across the globe (Garipcin and Seker, 2015). Current management practices employed in dairy farms for milk production might

be contributing factors associated with the dissemination of antibiotic-resistant bacterial strains. Our investigation had some limitations; we did not conduct molecular studies to detect antibiotic resistance genes especially for MRSA and MDRSA isolates due to financial restrictions. Therefore, it is suggested that molecular methods should be used to characterize these isolates for the presence of antibiotic resistance determinants which may provide data to support conclusions.

CONCLUSIONS

The present study revealed that nasal colonization of multidrug resistant *Staphylococcus aureus* isolates is prevalent among dairy farm workers in the study area. Duration of animal contact, gender and milking hygiene scores significantly associated with nasal carriage *Staphylococcus aureus* infection. Nasal carriage of *S. aureus* in people's noses plays an important role in the epidemiology and pathogenesis of infections caused by *S. aureus*. The high level of nasal carriage multi drug resistant *Staphylococcus aureus* isolates and prevalence of resistance against commonly used antimicrobials are, therefore, warrants judicious use of antimicrobials accompanied by strategies for prevention of spread of MDRSA. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

Conflict of Interest

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Diriba Lema, Dr. Birihanu Abera, Iyob Iticha and Tsion Bileta, for their encouragement, material support and information provision while collecting nasal swab samples from dairy farm workers. We also express our deepest gratitude and appreciation to Asella regional laboratory staff members for providing us valuable support and assistance during sample processing in the laboratory. We extend sincere appreciation to the dairy farm workers who participated in our study.

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